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EXAMINER	
SAOUD, CHRISTINE J	
ART UNIT	**PAPER NUMBER**
1647	

DATE MAILED: 10/17/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/905,348	ASHKENAZI ET AL.	
	Examiner	Art Unit	
	Christine J. Saoud	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 July 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *44-46 and 49-51* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *44-46 and 49-51* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *07/27/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114 was filed in this application after appeal to the Board of Patent Appeals and Interferences, but prior to a decision on the appeal. Since this application is eligible for continued examination under 37 CFR 1.114 and the fee set forth in 37 CFR 1.17(e) has been timely paid, the appeal has been withdrawn pursuant to 37 CFR 1.114 and prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on 27 July 2006 has been entered.

Response to Amendment

Claims 44-46 and 49-51 are pending in the instant application. Claims 1-43 and 47-48 have been canceled in a previous amendment.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Any objection or rejection of record which is not expressly repeated in this action has been overcome by Applicant's response and withdrawn.

Applicant's arguments filed 27 July 2006 have been fully considered but they are not deemed to be persuasive.

Information Disclosure Statement

The information disclosure statement (IDS) filed 27 July 2006 has been considered by the examiner.

Claim Rejections - 35 USC § 101

The Declaration under 37 CFR 1.132 filed 27 July 2006 has been received and fully considered. However, it is insufficient to overcome the rejection of claims 44-46 and 49-51 based upon lack of utility and enablement as set forth in the last Office action for the reasons provided below.

Claims 44-46 and 49-51 stand rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well-established utility for the reasons of record in the previous Office action(s).

Applicant argues (page 3) that it is not a legal requirement to establish a necessary correlation between an increase in the copy number of the mRNA and protein expression levels that would correlate to the disease state or evidence that protein levels can be accurately predicted. Applicant further argues that the evidentiary standard is a preponderance of the totality of the evidence under consideration. Applicant concludes that the question is whether it is more likely than not that a person of ordinary skill in the pertinent art would recognize such a positive correlation between gene copy number and protein expression levels. Applicant's arguments have been fully considered, but are not persuasive. Applicant's assessment of the question to be

asked is on point and correct. However, a review of the totality of the evidence under consideration must result in the conclusion that one skilled in the art would reasonably doubt the existence of a positive correlation between protein expression and gene copy number. While one can find prior art that supports a "reasonable probability" that mRNA and protein levels will correlate for a *given* protein, there is influential prior art of record that requires the Examiner maintain that, as a whole, the prior art does not provide a reasonable expectation that expression of the nucleic acid of SEQ ID NO:17 positively correlates with the expression of the protein of SEQ ID NO:18. The advent of proteome analysis has only recently begun to elucidate the reality of nucleic acid and protein expression which is becoming recognized as more complicated and different from the previously accepted dogma. It is also noted that the information on which the assumption of a correlation between mRNA and protein levels was based came from findings in normal, noncancerous tissue. Indeed, there is evidence in the art to refute generalizations about gene/protein correlations even in normal tissue. For example, Haynes et al. (Electrophoresis 19 : 1862-1871,1998) as discussed above showed from studies with yeast that among 80 proteins studied which were relatively homogenous in half-life and expression level, no strong correlation existed between protein and transcript levels. It was concluded that the protein levels cannot be accurately predicted from the level of the corresponding mRNA transcript (page 1863, second paragraph, and Figure 1). In a separate comparison by Fessler et al. (J. Biol. Chem. 277(35): 31291-302, Aug. 2002) examining lipopolysaccharide-activated neutrophils (col. 2, beginning of last paragraph on page 31300) it is stated, "Parallel use of DNA

microarrays and proteomics affords a powerful strategy for comparison of corresponding mRNA transcripts and proteins, thereby affording new insights into mechanisms by which the cell regulates its signaling response to the external environment. Of interest, a poor correlation was also found between corresponding transcripts and proteins (Table VIII), as reported in other systems." Fessler et al. warn (first sentence page 31296), "Nevertheless, the reliance upon DNA microarrays alone affords insight only into the transcriptional response without corroboration at the protein levels." Chen et al. (Mol. Cell Proteomics 1 .4:304, 2002) studied 165 proteins from lung adenocarcinoma tumors expressed by 98 individual genes. Their findings provide further evidence that one cannot assume the level of mRNA will correlate with the level of expressed protein for any given gene or any given protein (paragraph bridging pages 312-313):

> The results of this study indicate that the level of protein abundance in lung adenocarcinomas is associated with the corresponding levels of mRNA in 17% (28 proteins) of the total 165 protein spots examined. This was substantially higher than the amount predicted to result from chance alone (which was 5.1) and suggest that a transcriptional mechanism likely underlies the abundance of these proteins in lung adenocarcinomas. We also demonstrated that the expression of individual isoforms of the same protein may or may not correlate with the mRNA, indicated that the separate and likely post-translational mechanisms account for the regulation of isoform abundance. These mechanisms may also account for the differences in the correlation coefficients observed between stage I and stage III tumors, indicating that specific protein isoforms show regulatory changes during tumor progression.

Further it was shown (page 309, col. 2, 5th line) that, "In addition to differences in the relationship between mRNA levels and protein expression among separate isoforms, some genes with very comparable mRNA levels showed a 24-fold difference in their protein expression. Genes with comparable protein expression levels also showed up to

a 28-fold variation in their mRNA levels." Chen showed that not only with mRNAs that encode a single protein but also with nucleic acids that encode multiple isoforms, only a minority of mRNAs showed a correlation in levels of expression with their encoded proteins. Given the unknown amount that mRNA copy number of PRO232 increased in normal tissue compared to tumors, and the evidence provided by Haynes et al., Hu et al., Fessler et al. and Chen et al., one skilled in the art would not have assumed that a small increase in mRNA copy number would correlate with significantly increased polypeptide levels. The level of increase of the encoding nucleic acid is not disclosed. One skilled in the art would have to do further research to determine whether or not the PRO232 polypeptide levels were significantly increased significantly in tumor samples compared to normal tissue. Such further research requirements make it clear that the asserted utility is not yet in currently available form, i.e., it is not substantial. This further experimentation is part of the act of invention and until it has been undertaken, Applicant's claimed invention is incomplete. The instant situation is directly analogous to that which was addressed in Brenner v. Manson, 148 U.S.P.Q. 689 (Sus. Ct, 1966), in which the court held that:

> "The basic quid pro quo contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility", "[u]nless and until a process is refined and developed to this point-where specific benefit exists in currently available form-there is insufficient justification for permitting an applicant to engross what may prove to be a broad field", and "a patent is not a hunting license", "[i]t is not a reward for the search, but compensation for its successful conclusion."

Note that the invention must have a specific and substantial utility. The literature cautions researchers from drawing conclusions based on small changes in transcript

expression levels between normal and cancerous tissue (see Hu et al.). Without more specifics about necessary sample size, expression level range for normal and tumor tissues, the specification has not provided the invention in a form that can be used without necessary further experimentation.

Applicant has submitted teachings from Alberts, B. (Molecular Biology of the Cell (3rd ed 1994 and 4th ed 2002)) and Lewin, B. (Genes VI 1997) to support the statements of Dr. Polakis (Polakis II declaration). Applicant also cites numerous references to emphasize that those of skill in the art would not be focusing on differences in gene expression between cancer cells and normal cells if there were no correlation between gene expression and protein expression (such as Zhigang et al., Meric et al. Orntoft et al., Wang et al., Munaut et al., etc.). Applicant asserts that changes in mRNA level generally lead to corresponding changes in the level of expressed protein. Applicant also contends that the references and the Polakis declaration establish that the accepted understanding in the art is that there is a reasonable correlation between changes in gene expression and the level of the encoded protein.

Applicant's arguments have been fully considered but are not found to be persuasive. While the Examiner acknowledges the teachings of Alberts and Lewin, which disclose that initiation of transcription is the most common point for a cell to regulate the gene expression, it is not the only means of regulating gene expression. For example, Alberts also teaches that there are a number of other controls that can act later in the pathway from RNA to protein to modulate the amount of protein that is

made, including translational control mechanisms and mRNA degradation control mechanisms (see Alberts 3rd ed., bottom of pg 453). Meric et al. states the following:

> "The fundamental principle of molecular therapeutics in cancer is to exploit the differences in gene expression between cancer cells and normal cells. [M]ost efforts have concentrated on identifying differences in gene expression at the level or mRNA, which can be attributable to either DNA amplification or to differences in transcription."

However, Meric et al. also goes on to state that gene expression is quite complicated, and is also regulated at the level of mRNA stability, mRNA translation, and protein stability (see page 971, Introduction). Meric et al. also teaches that there are a number of translation alterations encountered in cancer, including variations in the mRNA sequence as a result of mutations, alternate splicing and transcription start sites, alternate polyadenylation sites, and alterations in the components of the translation machinery (see pages 973-974).

Furthermore, all of Applicant's newly cited references (with the exception of Orntoft et al.) measure mRNA with assays other than microarray, which is the assay utilized in Example 16 of the instant specification. Also, with the exception of Futcher et al., all of Applicant's newly cited references are directed to the analysis of single genes, or a small group of genes, and therefore do not demonstrate trends found across proteins in general. The studies cited by Applicant that examine the expression of specific genes or small numbers of genes are not found persuasive in view of comprehensive studies where significantly larger numbers of transcripts and proteins were examined and more accurately describe general trends, specifically, Haynes (80 proteins examined) and Chen (165 proteins examined) (cited previously by Examiner).

With regard to the Orntoft reference, Applicant argues at page 6 that Orntoft examined 40 well-resolved abundant proteins, and found significant correlation between mRNA and protein alterations (including both increases and decreases) for each gene, except one. Applicants' arguments with respect to Orntoft have been fully considered but are not found to be persuasive. Orntoft et al. appear to have looked at increased DNA content over large regions of chromosomes and compare that to mRNA and polypeptide levels from the chromosomal region. Their approach to investigating gene copy number was termed CGH. Orntoft et al. do not appear to look at gene amplification, mRNA levels and polypeptide levels from a single gene at a time. Orntoft et al. concentrated on regions of chromosomes with strong gains of chromosomal material containing clusters of genes (pg 40). This analysis was not done for PRO232 in the instant specification. That is, it is not clear whether or not PRO232 is in a gene cluster in a region of a chromosome that is highly amplified. Therefore, the relevance, if any of Orntoft et al. is not clear.

Applicant also asserts (page 10 of response) that Futcher et al. (1999) conducted a study of mRNA and protein expression in yeast and report a good correlation between protein abundance, mRNA abundance, and codon bias. Applicant's arguments have been fully considered but are not found to be persuasive. Futcher et al concludes that "[t]his validates the use of mRNA abundance as a rough predictor of protein abundance, at least for relatively abundant proteins [emphasis added]" (pg 7368, col 1). Futcher et al. also admits that Gygi et al. performed a similar study and generated similar data, but

reached a different conclusion. Futcher et al. indicates that "Gygi et al. feel that mRNA abundance is a poor predictor of protein abundance" (pg 7367, col 1, 1st full paragraph).

The Examiner maintains the previous argument of record, namely that mRNA levels are not necessarily predictive of protein levels even when there is a change in the mRNA level. Comprehensive studies where significantly large numbers of transcripts and proteins were examined report that increases in mRNA and protein samples are not correlated. Nagaraja et al. (Oncogene, 25:2328-2338, 2006) characterized comprehensive transcript and proteomic profiles of cell lines corresponding to normal breast (MCF10A), non-invasive breast cancer (MCF7) and invasive breast cancer (MDS-MB-231 and report that "the proteomic profiles indicated altered abundance of fewer proteins as compared to transcript profiles" (see abstract), and "the comparison of transcript profiles with proteomic profiles demonstrated that altered proteins were not always represented in the microarray designated profiles and *vice versa*" (see pg 2329, first column). Nagaraja et al. further report that, "a comparative analysis of transcripts and proteins to establish a relationship between transcript changes and protein levels has not yet become routine" (see pg 2328, second column). Lastly, Nagaraja et al. report that, "as dictated by post-transcriptional regulation, protein profiles showed far fewer changes as compared to transcript profiles" (see pg 2335, first column).

Similar results were reported by Waghray et al. (Proteomics, 1:1327-1338, 2001). Waghray et al. analyzed gene expression changes induced by dihydrostesterone (DHT) in the androgen responsive cancer line LNCaP, at both RNA and protein levels (see abstract). In this study, Waghray et al. identified transcripts from 16750 genes and

found 351 genes were significantly altered by DHT treatment and the RNA level, and identified 1031 proteins and found 44 protein spots that changed in intensity (either increased or decreased). Out of the 44 protein spots that changed in intensity, Waghray et al. reports that, "remarkably, for most of the proteins identified, there was no appreciable concordant change at the RNA level" (see pg 1333-1334, Table 4). Waghray et al. clearly state that, "The change in intensity for most of the affected proteins identified could not be predicted based on the level of the corresponding RNA" (see abstract).

In a review of gene expression in colorectal cancer (CRC), Sagynaliev et al. (Proteomics, 5:3066-3078, 2005) report that "it is also difficult to reproduce transcriptomics results with proteomics tools. Out of 982 genes found to be differentially expressed in human CRC by genome-wide transcriptomics technologies (Table 6a), only 177 (18%) have been confirmed using proteomics technologies" (see pg 3068).

In summary, it is clear that Nagaraja, Waghray and Sagynaliev support the Examiner's position that *changes* in mRNA expression frequently do not result in *changes* in protein expression. It is also noted that the specification of the instant application does not teach a change in mRNA level of PRO232. The specification simply discloses a static measurement of PRO232 mRNA in lung and colon tumor cells as compared to a universal control. There are no teachings in the specification as to the differential expression of PRO232 mRNA in the progression of lung or colon tumors in response to different treatments of hormones (for example). Therefore, the Examiner maintains that Applicant's measurement of an increase of PRO232 mRNA does not

provide a specific and substantial utility for the encoded protein, or an antibody to the protein.

The state of the art, as evidenced through textbooks and review papers, clearly establishes that polypeptide levels cannot be accurately predicted from mRNA levels. Lilley et al. teach that "DNA chips (mRNA profiling studies) can contribute to the study of gene expression in response to a particular biological perturbation. However, the extrapolation that changes in transcript level will also result in corresponding changes in protein amount or activity cannot always be made" ("Proteomics" Molecular Biology in Cellular Pathology, (2003) England: John Wiley & Sons, page 351). Wildsmith et al. also disclose that the gene expression data obtained from a microarray may differ from protein expression data ("Gene Expression Analysis Using Microarrays" Molecular Biology in Cellular Pathology, (2003) England: John Wiley & Sons, pages 269-286, especially pg 283). King et al. disclose that "it has been established that mRNA levels do not necessarily correlate with protein levels" (pg 2287, 2nd full paragraph). King et al. state that it has been demonstrated that correlation between mRNA and protein abundance is less than 0.5 and that "mRNA expression studies should be accompanied by analyses at the protein level" (pg 2287, bottom of col 1 through the top of col 2; see also Bork et al., Genome Res 398-400, 2000, especially pg 398, bottom of col 3). Haynes et al. teach that "[p]rotein expression levels are not predictable from the mRNA expression levels" (pg 1863, top of left column) and "only the direct analysis of mature protein products can reveal their correct identities, their relevant state of modification and/or association and their amounts" (pg 1870, under concluding remarks). Madoz-

Gurpide et al. disclose that "[f]or most of the published studies it is unclear how well RNA levels reported correlate with protein levels" (pg 53, 1st full paragraph).

However, the specification of the instant application has only disclosed that the PRO232 polynucleotide is overexpressed in lung and colon tumor cells. The specification does not indicate that the PRO232 polypeptide has been overexpressed in the lung or colon tumor samples tested. Given the asserted increase in PRO232 expression, and the evidence provided by the current literature, it is clear that one skilled in the art would not assume that an increase in mRNA expression would correlate with significantly increased polypeptide levels. Further research needs to be done to determine whether the purported increase in PRO232 DNA supports a role for the peptide in the cancerous tissue; such a role has not been suggested by the instant disclosure. Such further research requirements make it clear that the asserted utility is not yet in currently available form, i.e., it is not substantial. This further experimentation is part of the act of invention and until it has been undertaken, Applicant's claimed invention is incomplete. As discussed in *Brenner v. Manson*, (1966, 383 U.S. 519, 148 USPQ 689), the court held that:

> "The basic quid pro quo contemplated by the Constitution and the Congress for granting a patent monopoly is the benefit derived by the public from an invention with substantial utility", "[u]nless and until a process is refined and developed to this point-where specific benefit exists in currently available form-there is insufficient justification for permitting an applicant to engross what may prove to be a broad field", and, "a patent is not a hunting license", "[i]t is not a reward for the search, but compensation for its successful conclusion."

Accordingly, the specification's assertions that the PRO232 polypeptides have utility in the fields of cancer diagnostics is not substantial.

Without more specifics about necessary sample size, expression level range for

normal and tumor tissues, the specification has not provided the invention in a form readily usable by the skilled artisan such that significant further experimentation is unnecessary. The importance of replication in microarray gene expression studies is also demonstrated by Lee et al. (Proc. Natl. Acad., USA, 97(18):9834-9839, 2000) who report that, "our results show that any single microarray output is subject to substantial variability" and "we recommend that at least three replicates be used in designing experiments using cDNA microarrays" (see pg 9834, second column). A single output yields numerous misclassifications, especially numerous false positives (Lee et al., bottom of pg 9838). The importance of replication in microarray gene expression studies is also important when one considers the problem of variations within "normal" gene expression levels as reported by King et al. (JAMA, 286(18):2280-2288, 2006). King et al. report that "a significant portion of microarray data variability for high- or medium-abundance mRNAs may simply be due to normal expression variations" and that "Several previous studies have used arbitrary 2-fold change criteria to define significant expression change. However, the 2-fold threshold has been shown to be statistically invalid even for duplicate experiments" (see pg 2284, first column).

However, the PRO232 gene and polypeptide of the instant application have *not* been associated with tumor formation or the development of cancer, nor have they been shown to be predictive of such. The specification merely demonstrates that PRO232 was purportedly overexpressed in two different cancer samples. No mutation or translocation of PRO232 has been associated with any type of cancer versus normal tissue. It is not known whether PRO232 is expressed in corresponding normal tissues,

and what the relative levels of expression are. In the absence of any of the above information, all that the specification does is present evidence that PRO6232 mRNA is amplified in two diverse samples and invites the artisan to determine the significance of this increase.

Applicant refers to the second declaration of Dr. Polakis (Polakis II), submitted with the instant response. Applicant argues that this declaration provides the facts, set forth in a table (Exhibit B), for independent evaluation by the Examiner. The second Polakis declaration under 37 CFR § 1.132 filed 27 July 2006 has been considered and is deemed insufficient to overcome the rejection of the claims based upon 35 U.S.C. §§ 101 and 112, first paragraph, for the following reasons. Specifically, data for PRO232 does not seem to appear in the table (Exhibit B). It is not clear which "UNQ" number refers specifically to PRO232, if any at all. Furthermore, it is not clear how the clones appearing in the table compare to PRO232, or if the results presented in the table were determined by the same methodology as presented in Example 16 of the instant specification. For example, how highly expressed were the genes in Exhibit B that purportedly correlate with increased protein levels, 2-fold, 5-fold, 10-fold? How many samples were used? By what means was the level of mRNA expression determined, e.g., microarray, Northern blot, quantitative PCR? Was the "universal normal control" used or were matched tissue controls used? The declaration only states that levels of mRNA and protein in tumor tissue were compared to normal tissue.

Additionally, the Examiner notes that the two Polakis declarations are not consistent. In the first declaration submitted 09 August 2004, Dr. Polakis declares that

"we have identified approximately 200 gene transcripts that are present in human tumor cells at significantly higher levels than in corresponding normal human cells". In the second declaration, he states that "we have identified approximately 200 gene transcripts that are present in human tumor *tissue* at significantly higher levels than in corresponding normal human *tissue*."

In the first declaration, Dr. Polakis declares that "In approximately 80% of our observations we have found that increases in the level of a particular mRNA correlates with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells." In the second, he states that "of the 31 genes identified as being detectably overexpressed in human tumor tissue as compared to normal human tissue at the mRNA level, 28 of them (i.e. greater than 90%) are also detectably overexpressed in human tumor tissue as compared to normal human tissue at the protein level." It cannot be determined whether the two declarations are referring to the same data set, or different data sets. Further, there has been no explanation of why the Declarant now refers to tumor *tissue* rather than tumor *cells,* nor what the perceived significance of this change is.

It is Applicant's position that the specification at, for example, Table 8 of Example 16, provides sufficient disclosure to establish a specific, substantial and credible utility for the PRO232 polypeptides. Applicant asserts that Examples 16 discloses that PRO232 is significantly overexpressed in various human tumor tissues as compared to a non-cancerous human tissue control. Applicant indicates that Table 8 explicitly states that PRO232 is significantly overexpressed in lung and colon tumor

cells as compared to universal normal control, and therefore PRO232 polypeptide could be used as a diagnostic tool.

Applicant's arguments have been fully considered but are not found to be persuasive. Specifically, in the instant case, the specification indicates overexpression of PRO232 mRNA in colon and lung tumor tissue (the numerical increase is not known). However, the specification fails to precisely disclose any correlation between the reported overexpression of PRO232 mRNA and PRO232 protein expression, and more importantly, to what extent PRO232 mRNA is reliably overexpressed in a particular tumor sample, such as colon, such that the PRO232 polypeptide encoded thereby could be used as a diagnostic marker for colon tumor cells. There is no evidence regarding whether or not PRO232 polypeptide levels are overexpressed in colon or lung tumor cells.

Specifically, although the "universal control" of the instant application is derived from tissues of epithelial origin (pg 121), there is no teaching in the specification that any epithelial samples were derived from colon. This issue is of importance because the specification asserts that the PRO232 nucleic acid is overexpressed in colon tumor cells, as well as lung tumor cells. It is not clear as to why the tumor tissues in Example 16 are not compared to single organ control samples. Additionally, the state of the art discloses that a standard normal is not so easy to define and gene expression in normal tissue is dependent upon several factors involving patient and sample variation (King et al., pg 2281, col 1, 2nd full paragraph). One complication encountered with microarray

expression profiles is that any given tissue is composed of many different cell types. For example, King et al. teaches that:

"a simple punch biopsy of the skin may contain keratinocytes, melanocytes, Langerhans cells, Merkel cells, adipocytes, smooth muscle cells of arrector pili, striated muscle cells of the panniculus carnosus, blood cells including immune system cells, and cellular elements of blood vessels, nerves, hair follicles, sebaceous glands, and sweat glands. Moreover, cells from each of these populations will be at various stages of development and levels of activation, performing different functions and responding to disease processes or treatments in different ways and to varying extents" (pg 2282, bottom of col 2 through the top of col 3).

Thus, pooling *different* non-cancerous human epithelial tissues for a "universal control" introduces variability in the microarray assay. Utilization of incorrect tissue for comparison (with the absence of, or diminished expression of a gene in a particular tissue) would artificially increase or decrease the magnitude of differences observed in the instant microarray. It is also noted that the specification of the instant application at pg 121 does not even indicate that the tissues used for the "universal control" were isolated from the same subject as the tumor sample subject. Again, using tumor and control samples from different subjects would introduce variability into the microarray assay, making it less comparable and accurate.

Additionally, it is well known in the art different tissues express different genes, that is what makes the liver different from the heart and the heart different from the brain. All tissues have the same genetic makeup, but gene expression is what dictates the function of particular tissues. In the instant case, proper controls are dependent on the tissue examined since tissue specific gene expression is a natural phenomenon. Saito-Hisaminato *et al.* demonstrate that among 23,040 genes studied in normal human tissue, 4080 genes were highly expressed (greater than 5-fold higher than other tissues)

in one, or only a few tissues (see abstract, lines 3-5). This represents about 18% of the total genes tested. Further, a clustal analysis showed that lung was not at all similar to kidney or liver (see Figure 2, page 40), which are all mixed together in the instant "universal control". Saito-Hisaminato et al. only disclose genes that are highly expressed in one tissue compared to other tissues (i.e. greater than 5-fold difference). Thus, all of the undisclosed tissue-specific differences between 2- and 5-fold would be confounding and may artificially increase or decrease the magnitude of differences observed in the instant microarray as well. With respect to the instant "universal control", according to Saito-Hisaminato et al. (Figure 1, adding up the uniquely expressed genes for kidney, liver, and lung), the combination of liver, kidney, and lung as a control would result in about 600 highly expressed genes that would not reasonably be expressed in an appropriate control, such as colon. Also, an equally confounding problem of using the wrong tissue for comparison is the absence of, or diminished expression of a gene in a particular tissue. Accordingly, this would artificially increase or decrease the magnitude of differences observed in the instant microarray as well.

At page 4 of the response, Applicant argues that Pennica et al., Hu et al., and Haynes et al. do not show that a lack of correlation between gene (DNA) amplification and elevated protein levels exists, in general. Applicant's arguments have been fully considered but are not found to be persuasive. This has been fully considered but is not found to be persuasive because Haynes et al. clearly state "[p]rotein expression levels are not predictable from the mRNA expression levels" (pg 1863, top of left

column) and "only the direct analysis of mature protein products can reveal their correct identities, their relevant state of modification and/or association and their amounts" (pg 1870, under concluding remarks). Feroze-Merzoug et al. (Cancer and Metastasis Rev 20: 165-171, 2001) disclose that "[t]he lack of correlation between mRNA and corresponding protein is evident even in low eukaryotic cells such as yeast. Therefore, it will be necessary to profile both mRNA and protein for a complete picture of how cells are altered during malignant transformation" (pg 168, col 1). Madoz-Gurpide et al. also disclose that "[f]or most of the published studies it is unclear how well RNA levels reported correlate with protein levels" (pg 53, 1st full paragraph). Lilley et al. teach that "DNA chips (mRNA profiling studies) can contribute to the study of gene expression in response to a particular biological perturbation. However, the extrapolation that changes in transcript level will also result in corresponding changes in protein amount or activity cannot always be made"(page 351). Wildsmith et al. also disclose that the gene expression data obtained from a microarray may differ from protein expression data (pg 283). King et al. disclose that "it has been established that mRNA levels do not necessarily correlate with protein levels" (pg 2287, 2nd full paragraph). Clearly, Haynes et al., Feroze-Merzoug, Madoz-Gurpide et al., Lilley et al., Wildsmith et al., and King et al. indicate that mRNA levels do not predict protein levels.

Regarding Hu et al., Applicant's arguments have been fully considered but are not found to be persuasive. The asserted utility for the claimed polypeptides is based on Applicant's assertion that increased mRNA production leads to increased protein production. Hu et al. analyzed 2286 genes that showed a greater than 1-fold difference

in mean expression level between breast cancer samples and normal samples in a microarray (p. 408, middle of right column) and discovered that, for genes displaying a 5-fold change or less in tumors compared to normal, there was no evidence of a correlation between altered gene expression and a known role in the disease. However, among genes with a 10-fold or more change in expression level, there was a strong and significant correlation between expression level and a published role in the disease. The instant specification does not disclose that PRO232 mRNA levels are expressed at 10-fold or higher levels compared with normal, matched tissue samples. Therefore, based on Hu et al., the skilled artisan would not reasonably expect that PRO232 protein can be used as a cancer diagnostic. Regarding Applicant's criticism of Hu et al.'s statistical analysis (page 13 of the previously filed Appeal Brief), Applicant is holding Hu et al. to a higher standard than their own specification, which does not provide proper statistical analysis such as reproducibility, standard error rates, etc. Regarding Applicant's criticism of Hu et al. as being limited to a specific type of breast tumor, Hu et al. is cited as one of several pieces of evidence that gene amplification in a tumor does not correlate with mRNA overproduction or protein overproduction. Applicant repeatedly tries to impugn references for being drawn to different genes than PRO232, or different types of cancers, but have provided no more "relevant", e.g. closer to the instant fact situation, data or references. Accordingly, the record must be judged for what the cited references teach. When viewed with the evidence of record as a whole, there is no correlation between gene overexpression, mRNA levels and protein levels. In view of the totality of the evidence, including the declarations submitted under

37 CFR 1.132 and the publications of record (Haynes et al., Hu et al., Chen et al., Gygi et al., Madoz-Gurpide et al., Lilley et al., Wildsmith et al., King et al., and Bustin et al.), the instant utility rejection is appropriate.

Applicant cites Wang, Munaut, Hui, Khal, Maruyama, Caberlotto, Misrachi, Stein, Gou, Futcher, Godbout, Papotti, Van der Wilt, Grenback, Shen, and Fu as supporting their position that overexpression of mRNA correlates with overexpression of protein. As stated previously, Applicant's newly cited references with the exception of Futcher et al., are directed to the analysis of single genes, or a small group of genes, and therefore do not demonstrate trends found across proteins in general.

While the vast majority of newly cited references are drawn to predictability of protein on the basis of mRNA amplification (and for reasons cited above do not merit further discussion), a single reference, that by Godbout, is pertinent to the issue at hand. However, the Examiner finds Applicant's interpretation of the reference to be erroneous. Far from teaching predictability for expression of PRO232 on the basis of a minor genomic amplification, the abstract of Godbout teaches "The DEAD box gene, DDX1, is a putative RNA helicase that is co-amplified with MYCN in a subset of retinoblastoma (RB) and neuroblastoma (NB) tumors and cell lines. Although gene amplification usually involves hundreds to thousands of kilobase pairs of DNA, a number of studies suggest that co-amplified genes are only overexpressed if they provide a selective advantage to the cells in which they are amplified." The protein encoded by the DDX gene *had been characterized* as being a putative RNA helicase, a type of enzyme that *would be expected to confer a selective advantage* to the cells in

which it (the DDX gene) was amplified. On page 21167, right column, first full paragraph, Godbout et al. state "*It is generally accepted that co-amplified genes are not over-expressed unless they provide a selective growth advantage to the cell* (48, 49). For example, although ERBA is closely linked to ERBB2 in breast cancer and both genes are commonly amplified in these tumors, ERBA is not overexpressed (48). Similarly, three genes mapping to 12q13-14 (CDK4, SAS and MDM2) are overexpressed in a high percentage of malignant gliomas showing amplification of this chromosomal region, while other genes mapping to this region (GADD153, GL1, and A2MR) are rarely overexpressed in gene-amplified malignant gliomas (50, 51). The first three genes are probably the main targets of the amplification process, while the latter three genes are probably incidentally included in the amplicons."

On the contrary, there is no structure/function analysis in the specification regarding the putative protein encoded by the PRO232 gene. It is not disclosed, and based upon the sequence searches in this case, the Examiner cannot find any reason to suspect, that the protein encoded by the PRO232 gene would confer any selective advantage on a cell expressing it. It has no known homology to an RNA helicase or any other protein that would be expected to confer a selective advantage to a tumor cell. Further, it cannot be determined from the abstract whether the level of genomic amplification of the DDX1 gene was comparable to that disclosed for PRO232.

In summary, of applicants 100+ references submitted, only a single one, Godbout, is drawn to the predictability of protein levels based upon genomic DNA amplification, and that one supports the Examiners assertion that it is more likely than

not that the PRO232 protein would *not* be expected to be found in increased amounts in the cells tested by Applicant, and thus has no utility as a cancer diagnostic.

An additional reference that provides evidence that gene amplification does not necessarily lead to increased transcript is Li et al., Oncogene, Vol. 25, pages 2628-2635, 2006. Li et al. used a functional approach that integrated simultaneous genomic and transcript microarray, proteomics, and tissue microarray analyses to directly identify putative oncogenes in lung adenocarcinoma. On page 2633, right column, Li et al. state: "*In our study, 68.8% of the genes showing over-representation in the genome did not show elevated transcript levels*, implying that at least some of these genes are 'passenger' genes that are concurrently amplified because of their location with respect to amplicons but *lack biological relevance in terms of the development of lung adenocarcinoma*."

For all these reasons, the rejection is maintained.

Claims 44-46 and 49-51 also stand rejected under 35 U.S.C. 112, first paragraph for the reasons of record in the previous Office action(s). Specifically, since the claimed invention is not supported by either a specific or substantial asserted utility or a well-established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

Priority Determination

As the claimed subject matter is found to lack utility and enablement under 35 U.S.C. §§ 101 and 112, first paragraph, respectively, the effective priority date for this application is the instant filing date, 13 July 2001. Applicant's belief that they are entitled to the filing date of September 17, 1997 is noted, but not persuasive in view of the rejections of record.

Claim Rejections - 35 USC § 102

Claims 44 and 46 stand rejected under 35 U.S.C. 102(b) as being anticipated by Rosenthal et al. (DE 19818619-A1, 28 October 1999) for the reasons of record in the previous Office action(s).

Applicant argues that the claimed priority of the instant application is 17 September 1997, and therefore, the rejection is not proper. This argument is not persuasive in light of the utility rejection and the effective priority of the instant application based on the lack of utility.

Conclusion

No claim is allowed.

This is a continuation of applicant's earlier Application No. 09/905,348. All claims are drawn to the same invention claimed in the earlier application and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the earlier application. Accordingly, **THIS ACTION IS MADE FINAL**

even though it is a first action in this case. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no, however, event will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine J. Saoud whose telephone number is 571-272-0891. The examiner can normally be reached on Monday-Friday, 6AM-2PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brenda Brumback can be reached on 571-272-0961. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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